Research Highlights

- Certain bacteria migrate along fungal hyphae, interacting closely with the fungus.
- Three fungus-migratory bacteria were isolated from wood-decay fungi.
- The bacteria migrated along 10 wood-decay fungal strains when grown in co-culture.
- Two *Phanerochaete* strains were significantly inhibited by the bacteria.
- Bacteria could alter the outcome of inter-fungal competitive interactions.

The influence of migratory *Paraburkholderia* on growth and competition of wood-decay fungi

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Running head: Migratory bacteria on wood-decay fungi

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Abstract

Certain bacteria are capable of migrating along fungal hyphae, using them as a dispersal mechanism to cross otherwise-prohibitory distances. Three strains of fungal-migratory *Paraburkholderia* were isolated from the mycelium of wood-decay fungi, and inoculated onto ten strains of wood-decay fungi growing on solid agar medium. Two of the three bacteria were able to migrate along the hyphae of all fungi, although to differing extents. No bacteria-associated growth inhibition was observed with eight of the ten fungi, but mycelial extension rate of two strains of *Phanerochaete* was significantly reduced. Bacteria were also introduced into fungus-fungus competitive pairings, and significantly reduced the competitive performance of one of the *Phanerochaete* strains. Additionally, in several cases, introducing bacteria into competitive interactions made the outcome unpredictable, whereas in the absence of bacteria one fungus was consistently dominant. This is the first time that bacteria have been shown to influence fungal inter-specific competition.

147 words

Keywords: fungi; bacteria; wood; migration; competition; *Paraburkholderia*; tripartite interactions

Introduction

The phenomenon of fungal-migratory bacteria has been recorded for decades (Leben 1984), but has only recently been investigated in detail. Certain bacterial strains have the ability to migrate along fungal hyphae to disperse further and in less favourable conditions than would otherwise be possible (Warmink & van Elsas 2009; Nazir *et al.* 2010). Fungal-migratory bacteria have been grouped into two types: single-strain migrators are capable of movement along hyphae when grown in pure culture with the fungus, whilst community migrators can only track along hyphae as part of a bacterial consortium (Warmink & van Elsas 2009; Warmink *et al.* 2011). It is important to note that migration requires active movement by the bacterium, as fungal hyphae extend apically whilst the rest of the hypha remains stationary in the substratum. Migratory bacteria have been isolated from a variety of soil types, from clay to sandy loam (Nazir *et al.* 2012). Soil type apparently influences migration competence; migration success along the same fungal host can vary depending on the soil they are in (Nazir *et al.* 2012). A single bacterial strain can migrate along multiple hosts, but each fungal species appears to have an individual 'carrying capacity' in terms of the bacterial cell count it can support (Nazir *et al.* 2014). Migration is frequently directional, with most bacteria moving preferentially towards the hyphal tips (Warmink & van Elsas 2009; Nazir *et al.* 2014), although others move in the opposite direction (Leben 1984; Hover *et al.* 2016; Yang *et al.* 2018).

The best-characterised migratory bacteria isolated to date have been assigned to the genus *Burkholderia*, although since the splitting of this genus they have been reassigned *Paraburkholderia* (Sawana *et al.* 2014; Oren & Garrity 2015). This genus seems to be especially predisposed to forming fungal symbioses of various kinds (Stopnisek *et al.* 2015; Johnston *et al.* 2016). *Paraburkholderia* spp. have relatively large genomes for bacteria, and produce a diverse range of secondary metabolites, some of which have anti-fungal activity (Depoorter *et al.* 2016). Genome analysis of the independent migrator *Paraburkholderia terrae* BS001 revealed motility-related genes for flagella and walking pili, as well as genes involved in biofilm formation (Haq *et al.* 2014). Intriguingly, when a migratory strain of *Paraburkholderia glathei* was grown axenically it expressed cell motility proteins, but when co-

cultured with a fungus many of these proteins were no longer expressed (Stopnisek *et al.* 2015); *P. terrae* BS001 likewise upregulated motility genes when near a fungus, but downregulated them again once hyphal contact had been made (Haq *et al.* 2017). A type III secretion system (T3SS) appears to have a beneficial but non-essential role in these migratory associations (Haq *et al.* 2016; Yang *et al.* 2016; Nazir *et al.* 2017).

The exact nature of this fungus-bacteria relationship remains unknown, in terms of the costs and benefits to each partner. The presence of a fungus allowed migratory bacteria to survive and grow in low pH soil and low-nutrient medium, neither of which could support the bacteria alone (Warmink & van Elsas 2009; Nazir *et al.* 2012; Stopnisek *et al.* 2015). The benefit to the bacteria could be mimicked in the absence of the fungus by raising the soil pH and adding glycerol (Nazir *et al.* 2010). There are also indications that these benefits come at a cost: *P. glathei* upregulated its stress responses when tracking along a fungus (Stopnisek *et al.* 2015), possibly indicating that these bacteria may experience antibiosis from the fungus. Some community-migratory bacteria are selectively inhibited by the fungus *Lyophyllum* sp. strain Karsten (Warmink & van Elsas 2009). Outcomes for the fungi present a mixed picture. *Paraburkholderia terrae* BS001 protects its hosts from inhibition by pathogenic bacteria and ambient anti-fungal compounds (Nazir *et al.* 2014). Conversely, *Serratia marcescens* migrating over *Rhizopus oryzae* kill the fungus (Hover *et al.* 2016).

Thus far, there has been little exploration of whether migratory bacteria occur with wood-decay fungi: much of the work has focused on soil saprotrophs (Nazir *et al.* 2014; Haq *et al.* 2016; Simon *et al.* 2017), and there has been no exploration of whether wood-decay fungi coexist with migratory bacteria in the field. This relationship would be particularly interesting for cord-forming fungi, which primarily disperse, not as spores, but by forming large networks of mycelial cords across the forest floor (Boddy & Hiscox 2016). These networks would represent a major dispersal opportunity for any bacterium capable of exploiting them, providing a favourable habitat along which to migrate, and direct passage to new resources. *Paraburkholderia terrae* BS001 has been

experimentally observed to migrate along the mycelial cords of *Phanerochaete velutina*, albeit less proficiently than with other fungal species (Nazir *et al.* 2014).

Wood-decay fungal community dynamics are driven by inter-specific competition in the form of direct, confrontational interactions (Boddy 2000). Wood-decay fungi frequently encounter one another as mycelia within a woody resource, or cord systems on the forest floor. When this happens, they engage each other via diffusible and volatile compounds, increased enzyme activity, environmental pH manipulation and gross mycelial contact (Boddy 2000). This has two implications for any bacterial symbionts living with wood-decay fungi. Firstly, these fungi are highly capable of manipulating the microbial community in their surroundings, and there is evidence that this extends beyond other fungi to include bacteria (Folman *et al.* 2008; Johnston *et al*., submitted). Secondly, there is potential for bacteria in turn to influence fungal communities by affecting the outcomes of interactions. Wood-decay fungi form competitive hierarchies, but these can be altered by changes in abiotic or biotic conditions. For example, invertebrate grazers can exert top-down control of fungal communities when they preferentially graze the dominant competitor, weakening it such that a less competitive but grazing-resistant fungus is able to take over (Crowther *et al.* 2011). In this manner, the presence of a 'controlling' organism can have knock-on effects throughout the dead-wood environment.

The present study investigates the relationship between wood-decay fungi and migratory bacteria. The first objective was to isolate migratory bacteria directly from wood-decay fungi, in a UK mixed deciduous woodland: to date, the best-studied strains originate from soil in the Netherlands. Obtaining these strains allowed three hypotheses to be tested: (a) that the bacteria would be competent to migrate with a range of different wood-decay fungi as hosts; (b) that the presence of migratory bacteria would reduce fungal extension rate; and (c) that the presence of migratory bacteria would affect the competitive ability of wood-decay fungi.

Methods

Overview

Migratory bacteria were isolated directly from the mycelium of wood-decay fungi. These bacteria were then used in agar-based laboratory experiments to determine their effect on fungal growth and competitive ability.

Isolation and identification of migratory bacteria from the field

Mycelial cords were collected from the forest floor at Whitestone Woods, Monmouthshire, U.K. in the autumn-winter of 2015 (lat. 51.72, long. -2.69; site described by Hiscox *et al.* (2016)). Cords were vigorously shaken by vortex-mixer in ten changes of sterile distilled water to remove all but tightly attached bacterial cells, before inoculation onto 2% malt agar (1.5 g l^{-1} LabM agar 1, 2 g l^{-1} malt). Static bacterial colonies were excluded during subsequent subcultures of the mycelium, but bacteria that clearly tracked the hyphal growth were retained. The fungus was thus used as the sole enrichment agent. Migratory colonies could be clearly identified, as they mapped exactly onto the hyphal growth pattern (Fig S1). Of the 53 cords collected, migratory bacteria were obtained only from one. Pure cultures were established of both the bacterium (*Paraburkholderia* sp. BCC1884) and fungus (*Phanerochaete* sp. PW271).

Two further strains of migratory bacteria (*Paraburkholderia* sp. BCC1885 and *Paraburkholderia* sp. BCC1886) were obtained from mycelium isolated from decaying beech wood (*Fagus sylvatica*) at the same site. Small chips of wood were taken under aseptic conditions from the interior of 3 x 3 x 3 cm blocks that had been colonised with *Vuilleminia comedens* in the laboratory, and left on the woodland floor for 84 days. The chips were placed onto 2% malt agar and bacteria isolated as above.

For preliminary identification of bacteria and fungi, DNA was extracted from cultures with the PowerSoil® kit (MO BIO, Carlsbad, USA) (amended to include 20 s at 4 m s^{-1} in a MPBio FastPrep bead beater). Fungal ITS rDNA markers were amplified using primers gITS7F (GTGARTCATCGARTCTTTG) and tagged ITS4R (TCCTCCGCTTATTGATATGC-AGTACGAG) (Ihrmark *et al.*

2012) in 50 µl reactions containing 2.5 µl template, 300 nM tagged ITS4, 500 nM gITS7, 0.025 U HS Taq polymerase (PCR Biosystems, UK) and 10 µl supplied buffer, in a Dyad DNA Engine Peltier thermal cycler. The initial incubation was 94°C for 5 min, followed by 24 cycles of 30 s at 94°C, 30 s at 56°C and 30 s at 72°C, with a final incubation at 72°C for 7 min. Bacterial 16S rRNA gene makers were amplified with primers 27F (AGAGTTTGATCMTTGGCTCAG) (Weisburg *et al.* 1991) and 1492R (TACCTTGTTACGACTT) (Lane *et al.* 1985) in 50 µl reactions containing 1 µl DNA, 200 nM each primer (MWG Eurofins, Ebersberg, Germany), 5 µg BSA (Promega, WI, USA), 0.025 U μl^{-1} Taq polymerase (PCR Biosystems, UK) and 10 µl supplied buffer, in a Dyad DNA Engine Peltier thermal cycler. The initial incubation was 95°C for 2 min, followed by 35 cycles of 94°C for 30 s, 52°C for 30 s, 72°C for 1.5 min increasing by 1 s $cycle^{-1}$, and a final incubation at 72°C for 5 min. The amplicons were purified with the QIAQuick gel extraction kit (Qiagen, Hilden, Germany), and sent for unidirectional Sanger sequencing by MWG Eurofins. Fungal sequences were assigned against the UNITE ITS database (Kõljalg *et al.* 2013) using BLASTn as a search engine. Bacterial sequences were assigned against the Greengenes database (DeSantis *et al.* 2006) in QIIME using UCLUST as a search engine (Caporaso *et al.* 2010).

Bacterial motility

Swimming and swarming assays were conducted to determine motility of *Paraburkholderia* in absence of fungi. The starting inocula were 48-hour old liquid cultures, all containing between 1.4-3.6 x 10^5 CFUs. A sterile cocktail stick was used to transfer a small amount of material directly in the centre of the plate to avoid running of inoculum. A sterile cocktail stick was dipped into liquid inoculum, and was either stabbed to the bottom of the centre of the plate (swimming assays) or gently touched onto the surface of the centre of the plate (swarming assays). Three biological replicates were used with 5 technical replicates each. Plates were poured the afternoon before inoculation to keep moisture content consistent. Standard agar concentrations were used of 0.3% purified agar for swimming assays and 0.5% purified agar for swarming assays. Both assays were repeated using 1.5% agar, the concentration used in the fungal medium. All media were

supplemented with 2% malt. Plates were incubated at room temperature, stacked in piles three plates high to avoid moisture loss. Readings were taken every day for 5 days. Measurement were taken across 4 radii and averaged to reach a mean for each plate.

Culture preparation and maintenance

All ten wood-decay fungi used in the experiments were taken from the Cardiff Culture Collection (Table 1). They were selected to represent a variety of successional stages and, therefore, competitive abilities. The strains represent a range of taxonomic dissimilarity, from inter-phylum to intra-specific. All fungi were maintained on 2% malt agar for the duration of the experiment.

The three migratory bacterial strains were maintained on 2% malt agar plates, where they showed weak to no motility in the absence of a fungus (either remaining static or showing limited swarming behaviour, insufficient to explain the distance they travelled on the fungal plates). Bacterial cultures for long-term storage were frozen at -80°C in 2% malt broth diluted 80:20 with glycerol. Experimental bacterial inoculum was prepared from 48-hr cultures grown at 20°C in 2% malt broth with gentle agitation. The suspension was diluted 1:5 in 2% malt broth on the morning of the set-up, and viable cell numbers determined by dilution series and total viable counting (TVC).

Mycelial extension rates

6 mm plugs were cut from 7-day old fungal cultures and individually inoculated onto 2% malt agar in 9 cm diameter Petri dishes. The following day, each was inoculated with a 10 µL drop of bacterial suspension (treatment; 2-7 x 10^5 colony-forming units, CFU) or sterile broth (control) directly on top of the inoculum plug. Plates were incubated at 10°C for 7 weeks or 20°C for 4 weeks. Five replicates were prepared per treatment. Mycelial extension and bacterial colony migration were measured along four radii extending from the edge of the inoculum plug. Colony migration was assessed visually by following the appearance of bacterial colonies on the agar in immediate contact with individual hyphae (Fig S1). While measuring visible colonies is inferior to reisolation-based methods (e.g. Zhang *et al.* 2018) for capturing the full extent of migration, it was necessary in this case to handle the large numbers of plates. To compare the two methods, we obtained reisolation

data for all three strains migrating over *P. velutina* and *S. hirsutum*, and for BCC1884 and BCC1885 migrating over *B. adusta*, using the method of Zhang *et al.* (2018). The extent to which bacterial were re-isolated ahead of the visible migration front was variable across bacterial strains and fungi, with no obvious pattern; however, the correlation coefficient between migration measured by observation and migration measured by reisolation was 0.81.

Competition experiments

Plates were prepared as for the growth rate experiment, except that two plugs were inoculated onto each plate 3.5 cm apart, to set up competitive interactions. A restricted range of fungi was used due to the large number of potential combinations. *Bjerkandera adusta*, *Stereum hirsutum*, *Trametes versicolor*, *Phanerochaete velutina* and *Phanerochaete* sp. PW271 were paired in all combinations, including self-pairings. For each pairwise combination of fungi, interactions were set up with bacteria on both competitors; bacteria on one competitor but not the other, and *vice versa*; and bacteria on neither competitor. Five replicates were prepared per treatment. Bacteria (1-7 x 10^4 CFUs) and sterile broth were added as for the growth rates experiment. Plates were incubated at 10°C for 8 weeks or 20°C for 6 weeks, and the progress of interactions recorded weekly by visual inspection. At the end of the experiment, fungal re-isolations were made from the underside of the agar disk to verify that any observed replacement was not merely overgrowth. Bacterial re-isolations were made by drawing a sterile loop across the surface and streaking onto 2% malt agar.

Statistical analysis

Unless otherwise stated, all analysis was performed in R (R Development Core Team 2011) using RStudio (RStudio Team 2016) and packages *dplyr* (Wickham & Francois 2016), *ggplot2* (Wickham 2009), *lattice* (Sarkar 2008), *lsmeans* (Lenth 2016) and *nlme* (Pinheiro *et al.* 2016). R code to reproduce the analyses is available as an R markdown at *github.com/ecologysarah/migratory-paraburkholderia-interactions* (Allaire *et al.* 2016).

Mycelial extension rates were modelled using a linear mixed effects model (Zuur *et al.* 2009), with fungal identity, bacterial identity, temperature and time as fixed main effects predicting the length of outgrowing mycelium. *Fungus : time* and *fungus : bacterium* interactions were also included, as each fungus grows at a different rate and may respond differently to the bacteria. The random effects part of the model consisted of a random intercept for replicate identity and a random slope for *time | replicate identity*, to account for the longitudinal design. A likelihood ratio test established the overall significance of the *fungus : bacterium* interaction, the main term of interest in the model. *Post-hoc* comparisons were done in package *lsmeans* with Tukey adjustment.

The outcomes of competitive interactions were codified as an interaction metric. Each competitor was given a score of 2 for completely replacing the other fungus; 1 for partial replacement; 0 for deadlock; -1 for being partially replaced; or -2 for being completely replaced. The number of weeks that each interaction took to reach that conclusion was recorded, and the metric calculated as *score x (1/time of completion)*. This gave an identical score of opposite sign to each competitor, and created a metric whereby a fast win scored higher than a slow one, and a fast loss scored lower than a slow one. Deadlock always produced a score of 0.

In each interaction, the fungal competitors were arbitrarily assigned as F1 and F2. The outcomes were modelled by regressing the metric score for F1 against a zero-sum contrast matrix in which F1 was coded as positive and F2 as negative. When F1 won, then its positive (winning) scores continued to be counted as positive, and were simultaneously multiplied by -1 and counted as negative to F2; when F1 lost, its negative (losing) scores continued to be negative, and were simultaneously multiplied by -1 and counted as positive to F2. The model then included a second predictor matrix with a column for each combination of fungus and bacterium, *e.g. Paraburkholderia* sp. BCC1884 on *B. adusta*. If F1 had been inoculated with bacteria, it received a 1 in the relevant column; if F2 had been inoculated, it received -1 in the relevant column. This allowed the effect of each bacterium on each fungus to be estimated irrespective of whether the fungus was F1 or F2. Finally, temperature was included in the model as a simple categorical variable.

Results

Bacterial strain identity

Strain BCC1884 was isolated from a mycelial cord of *Phanerochaete* sp. PW271 (the only migratory strain to be obtained out of 53 cord samples), whilst strains BCC1885 and BCC1886 were isolated serendipitously from *F. sylvatica* wood blocks being decayed by *V. comedens*. 16S rRNA gene sequencing placed all three strains within the genus *Burkholderia*, as the Greengenes database does not currently reflect the updated *Paraburkholderia* taxonomy. Subsequent whole-genome analysis placed the strains within *Burkholderia* clade 3 (Webster *et al.* 2019), which has been reassigned to the new genus *Paraburkholderia* (Sawana *et al.* 2014). BCC1884 clustered most closely to *Paraburkholderia bryophila*, BCC1885 to *Paraburkholderia terricola and Paraburkholderia sediminicola* and BCC1886 to *Paraburkholderia phenazinium* (Webster *et al.* 2019).

Migratory capability of bacterial isolates

In the absence of mycelium, all isolates showed some motility in swarming and swimming assays (Fig S2). BCC1886 was the most motile, and BCC1885 the least. However, for all three strains motility was negligible when growing on 1.5 % agar in the absence of mycelium (Fig S2).

All the bacteria were competent to migrate along at least one fungus, and all fungi successfully hosted at least one bacterium (Fig 1). There was considerable inter-fungal variation in the success of bacterial establishment and the extent of colony migration (Fig 1). Bacteria travelled furthest on the two *Phanerochaete* strains, and also migrated very successfully on *S. hirsutum*. In contrast, only one replicate indicated substantial bacterial colony migration on *R. bicolor*. The two strains of *H. fasciculare* showed an unexpected intra-specific difference: bacteria successfully established on only 5 out of a total of 30 plates of *H. fasciculare* GtWV2 (3 bacterial strains x 2 temperatures x 5 replicates), compared with 19 out of 30 for *H. fasciculare* ABWS1. The only bacterium-fungus combination that completely failed to establish was BCC1885 on *H. fasciculare* GtWV2.

Mycelial extension rates

As expected, fungi showed considerable inter-specific variation in extension rate. At 20°C the fastest species, *B. adusta*, averaged 6.0 mm day^{-1}, whilst the slowest grower, *H. fasciculare* ABWS1, averaged 1.1 mm day^{-1} (Fig 2a). At 10°C, growth rates were reduced for all fungi, typically 2-3 times slower than at 20°C (Fig 2b) ($t = 18.7$, $P < 0.001$).

None of the three bacterial treatments significantly altered fungal extension rate when considered across all fungi. However, there was a significant interaction between fungal identity and bacterial treatment (LR 136, $P < 0.001$). *Post-hoc* comparisons revealed that the interaction was driven by the *Phanerochaete* species, both of which showed significantly slower growth in the presence of bacteria (Table 2). The only other significant effect was on *H. fasciculare* ABWS1, where the mycelial extension rate increased by 28% in the presence of *Paraburkholderia* sp. BCC1886.

Effects of bacteria on inter-fungal competitive interactions

In the controls (*i.e.* when bacteria were absent), *B. adusta* had the highest average interaction score, followed by *S. hirsutum*, *T. versicolor*, *Phanerochaete* sp. and *P. velutina*. The presence of bacteria significantly affected fungal performance in a species-specific fashion (Table 3). *Phanerochaete* sp. PW271 had significantly reduced performance scores when inoculated with any of the three bacteria. *Paraburkholderia* sp. BCC1885 also significantly reduced the performance of *B. adusta* and *P. velutina*, although to a lesser extent. In some cases the addition of bacteria could reverse the outcome of the interaction (*e.g. T. versicolor vs. Phanerochaete* sp. and *P. velutina vs. Phanerochaete* sp. at 20°C; Fig 3); there were also instances where one fungus consistently dominated when bacteria were absent, but the addition of bacteria rendered the competitive outcome unpredictable (*e.g. B. adusta vs. P. velutina* at 10°C, *P. velutina vs. T. versicolor* and *P. velutina vs. S. hirsutum* at 20°C; Figs 3 & 4). All the fungal self-pairings met and merged as expected, regardless of bacterial treatment.

Observations on fungal morphology and bacterial behaviour

The presence of bacteria induced highly localised pigment production in *S. hirsutum* (Fig 5); the progress of bacterial colony migration was marked by bright orange colouration. Bacterial establishment on mycelium was often asymmetric, resulting in parts of the mycelium being colonised and others remaining (visually) bacteria-free. Bacteria were sometimes observed to cross the interaction front and become established on the other fungus. This indicates that whilst migration preferentially occurred towards the hyphal tips, there was at least some capacity to move in the opposite direction.

Discussion

This is the first report of migratory bacteria isolated from wood-decay fungi, and indicates that they co-occur naturally both in wood and on mycelial cords. Whilst most fungi appeared unaffected by hosting bacteria, active fungal-bacterial interaction was revealed by growth and competitive inhibition of two strains of *Phanerochaete*, and by morphological responses in *S. hirsutum*. This is the first assessment of how bacteria influence fungal inter-specific interactions, and reveals that they consistently impede the performance of some strains. The addition of bacteria can also change or destabilise the outcome of interactions in a pairing-specific fashion.

All three of the bacterial strains isolated were from a single clade of the proposed genus *Paraburkholderia*, even though the methodology used was not designed specifically to enrich this particular taxon. This provides further evidence to suggest that *Burkholderiaceae* is an important fungal-associated family, and *Paraburkholderia* species, in particular, are important among fungal-migratory bacteria (Nazir *et al.* 2012; Stopnisek *et al.* 2015; Johnston *et al.* 2016; Simon *et al.* 2017). Phylogenomic analysis of the three isolated bacterial strains (Webster *et al.* 2019) has indicated that all three fall within a single clade, closely related to *P. fungorum, P. terricola, P. xenovorans* and *P. terrae*. This clade also includes many migratory or otherwise fungal-associated species. *P. fungorum* was originally isolated from a wood-decay fungus, *Phanerochaete chrysosporium*, with which it

formed close hyphal associations (although whether *P. fungorum* migrated along the host was not reported) (Seigle-Murandi *et al.* 1996; Coenye *et al.* 2001). *P. terricola* and *P. xenovorans* are both capable of single-strain migration on *Lyophyllum* sp. strain Karsten (Nazir *et al.* 2012). *P. terrae* is the best characterised of all migratory bacteria, with multiple strains known to migrate on a range of fungi (Nazir *et al.* 2012; Haq *et al.* 2014; Nazir *et al.* 2014). The sequencing and analysis of *Paraburkholderia* spp. genomes (e.g. Haq *et al.* 2014; Webster *et al.* 2019), opens the door to future comparison between *P. terrae* and the present strains isolated in this study that may reveal a genomic signature of migration. Previous work has pointed to flagella as the major enablers of migratory behaviour, with type III secretion systems and pili also likely to play a minor role (Yang *et al.* 2016; Yang *et al.* 2017; Nazir *et al.* 2017).

Both the extension rate and competition experiments were conducted at 10°C and 20°C, to check that results were consistent across temperatures. Visually, temperature appeared to alter the relationship in some instances (such as the effect of bacteria on *B. adusta* extension rate; Fig 2) however, due to the complexity of the models it was not computationally feasible to test statistically for interactions between temperature and other predictors. Nonetheless, including data from both temperatures in the model showed that the patterns observed were large enough to remain when averaged across temperatures.

The asymmetric bacterial growth patterns must have been due to some micro-scale differences in conditions across the inoculation plug, but the nature of those differences can only be speculated on. It may have been due to a slight decentralisation of the inoculum drop, imperfections in the surface of the agar plug or subtle differences in the hyphae. Hyphal growth over the inoculum plug typically prevented immediate central dispersion of bacteria around the inoculum site, so the drop itself could have moved from the centre before drying. Whether fungal competitive ability is altered only on the sections of mycelium with active bacterial growth, or whether the bacteria produce systemic changes across the mycelium, is an interesting question that could be explored in future work.

Migratory bacteria occur naturally with wood-decay fungi and have a broad host range

Migratory bacteria were isolated from mycelial cords, but the retrieval rate was nonetheless low. The strains isolated from wood were discovered serendipitously, so detection rates cannot be compared. Nazir *et al.* (2014) reported low rates of migration over cords compared to hyphae, suggesting that cords may represent a more challenging venue for bacteria. Mycelial cords are toughened dispersal organs, aggregations of hyphae organised within a protective rind (Boddy 1993). This may provide the opportunity for migration of bacteria over the surface of the cord, and/or along hyphae inside the rind. Living within the rind would afford the bacteria direct access to the hyphae, and protection from the environment. Note that would not necessarily be endosymbiosis, as bacteria could be within the cord but not inside the hyphae.

The three strains of *Paraburkholderia* obtained were generalist migrators, capable of becoming established on multiple different fungi and thus supporting hypothesis 1. Each of the bacterial strains was able to migrate on a range of fungal hosts, representative of the species that would be encountered within their habitat. Two of the three bacteria (BCC1884 and BCC1886) migrated over all ten fungi (although with varying success), irrespective of fungal taxonomic position or ecological strategy.

Wood-decay fungi show inter- and intra-specific variation in their responses to migratory bacteria

Most of the fungi tested were neither inhibited nor stimulated by the presence of migratory *Paraburkholderia*, in accord with previous work (Nazir *et al.* 2014). Sometimes, this was because bacteria were largely unable to establish on the mycelium (*e.g. H. fasciculare* GtWV2). The difference in bacterial establishment between the two *H. fasciculare* strains was both unexpected and unexplained, but it indicates that the factors required for colonisation by migratory bacteria can vary at the intra-specific level. *R. bicolor* supported very limited bacterial movement, perhaps related to the calcium oxalate crystals which cover the hyphae (Connolly & Jellison 2011). Bacteria colonised and migrated along *S. hirsutum* very successfully, yet with no effect on its mycelial

extension rate – although its abundant pigmentation shows that it actively responded to bacterial presence.

The two *Phanerochaete* strains were notable exceptions to this general lack of growth response. Both provided a very successful platform for bacterial colony migration, and both were markedly inhibited in the process, thus offering partial support for the second hypothesis. Curiously, Nazir *et al.* (2014) used the same strain of *P. velutina* as the present study, and found it was unaffected when growing across soil with *Paraburkholderia terrae* BS001. This may reflect the difference in bacterial identity, venue of interaction (soil *vs.* agar) or fungal morphology (cords *vs.* hyphae). Although the present study contrasts with *P. terrae*'s lack of effect on host extension rate during co-migration, *P. terrae* has been observed to reduce fungal growth prior to physical contact (Haq *et al.* 2016).

Migratory bacteria can reduce the competitive ability of wood-decay fungi

As with extension rate, bacterial effects on competitive ability were fungus-specific, and so the third hypothesis was likewise partially supported. Once again, *Phanerochaete* sp. was the most affected, with a reduction in competitive ability when inoculated with any of the three bacteria. This fungus was isolated alongside *Paraburkholderia* sp. BCC1886, and it is noteworthy that the fungus most affected by bacteria was known to naturally host migratory bacteria. It is possible that this strain is particularly prone to exploitation by bacteria. Migratory bacteria are able to partner with a range of fungal strains and species, yet with a varying strength of interaction, mediated by different mechanisms (Nazir *et al.* 2014; Haq *et al.* 2016). The negative effects on both growth and competition suggest that these *Paraburkholderia* may be parasites, dispersing along fungal hyphae whilst disadvantaging the host. Conversely, *P. terrae* BS001 can protect its host from harmful bacteria and anti-fungal compounds (Nazir *et al.* 2014). Future work on the strains from the current study should investigate whether they also exhibit this ability, in which case there may be a fitness trade-off for fungi between the cost of hosting migratory bacteria and the protection that they afford. Wood-decay fungi exert active selection over the bacteria in their resource (Folman *et al.*

2008; Johnston *et al*., submitted), so the persistence of *Paraburkholderia* on mycelium indicates that it must be either tolerated by the fungus or resistant to its anti-bacterial mechanisms.

Wood-decay fungi form competitive hierarchies, whereby one species will outcompete another in a predictable (though not deterministic) manner (Boddy 2000). The competitive hierarchy observed in the present study was reversed compared to that normally expected (Hiscox *et al.* 2017), with the earlier coloniser *B. adusta* scoring highest and the later-stage *Phanerochaete* strains scoring lowest. On a rich agar medium, fast-growing earlier-successional species are at an advantage, which probably explains why they were able to outcompete the slower-growing later colonisers. This may also help to explain the reduced performance of *Phanerochaete* sp., as the bacteria slow its growth rate even further.

In some combinations, the addition of the bacteria reversed the outcome of the interaction (*e.g. T. versicolor vs. Phanerochaete* sp. and *P. velutina vs. Phanerochaete* sp. at 20°C), or destabilised an otherwise predictable relationship (*e.g. P. velutina vs. T. versicolor* and *P. velutina vs. S. hirsutum* at 20°C). The effect is reminiscent of how grazing invertebrates can reverse interaction outcomes by preferentially consuming the stronger competitor (Crowther *et al.* 2011). Likewise, the addition of a third fungal competitor into a pairwise interaction can shift the outcome from consistent to unstable (Hiscox *et al.* 2017). These examples underline the importance of considering competition not only in terms of the competitors' own traits, but also as a process under cross-kingdom, top-down influences.

The addition of bacteria did not impede fungal self-recognition mechanisms, as all self-pairings showed normal recognition and mycelial fusion. Altering the bacterial community associated with the zygomycete *Mucor hiemalis* interfered with this process, producing antagonism between the cured and uncured mycelium (Schulz-Bohm *et al.* 2017). *Pseudomonas syringae* activates programmed cell death (and thus nutrient release) in *Neurospora crassa* by tapping into a somatic incompatibility pathway used in self-recognition (Wichmann *et al.* 2008).

Conclusions

In conclusion, migratory bacteria have been isolated directly from fungal mycelium, in a different habitat and geographically distant location to previous strains. These *Paraburkholderia* are competent to migrate with a taxonomically and ecologically diverse range of wood-decay fungi, which in turn showed species- and strain-specific responses to bacterial presence. Most fungi were largely unaffected in both growth and competitive ability, but the two *Phanerochaete* strains were consistently inhibited by the bacteria. These results show that wood-decay fungi naturally host migratory bacteria, and that these bacteria may influence the results of fungus-fungus interactions.

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References

Allaire, J., Cheng, J., Xie, Y., McPherson, J., Chang, W., Allen, J., Wickham, H., Atkins, A. & Hyndman, R. 2016. *rmarkdown*: Dynamic Documents for R.

Boddy, L. 2000. Interspecific combative interactions between wood-decaying basidiomycetes. *FEMS Microbiology Ecology* 31, 185–194.

Boddy, L. 1993. Saprotrophic cord-forming fungi: warfare strategies and other ecological aspects. *Mycological Research* 97, 641–655.

Boddy, L. & Hiscox, J. 2016. Fungal Ecology: Principles and Mechanisms of Colonization and Competition by Saprotrophic Fungi. *Microbiology Spectrum* 4, FUNK-0019-2016.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J. & Knight, R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7, 335–336.

Coenye, T., Laevens, S., Willems, A., Ohlen, M., Hannant, W., Govan, J.R.W., Gillis, M., Falsen, E. & P, V. 2001. Burkholderia caledonica sp . nov ., two new species isolated from the environment , animals and human clinical samples. *International Journal of Systematic and Evolutionary Microbiology* 51, 1099–1107.

Connolly, J.H. & Jellison, J. 2011. Calcium translocation, calcium oxalate accumulation, and hyphal sheath morphology in the white-rot fungus *Resinicium bicolor*. *Canadian Journal of Botany* 73, 927–936.

Crowther, T.W., Boddy, L. & Jones, T.H. 2011. Outcomes of fungal interactions are determined by soil invertebrate grazers. *Ecology Letters* 14, 1134–1142.

Depoorter, E., Bull, M.J., Peeters, C., Coenye, T., Vandamme, P. & Mahenthiralingam, E. 2016. *Burkholderia*: an update on taxonomy and biotechnological potential as antibiotic producers. *Applied Microbiology and Biotechnology* 100, 5215–5229.

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P. & Andersen, G.L. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology* 72, 5069–72.

Folman, L.B., Klein Gunnewiek, P.J., Boddy, L. & de Boer, W. 2008. Impact of white-rot fungi on numbers and community composition of bacteria colonizing beech wood from forest soil. *FEMS Microbiology Ecology* 63, 181–91.

Haq, I.U., da Rocha Calixto, R.O., Yang, P., dos Santos, G.M.P., Barreto-Bergter, E. & van Elsas, J.D. 2016. Chemotaxis and adherence to fungal surfaces are key components of the behavioral response of *Burkholderia terrae* BS001 to two selected soil fungi. *FEMS Microbiology Ecology* 92, fiw16.

Haq, I.U., Graupner, K., Nazir, R. & van Elsas, J.D. 2014. The Genome of the Fungal-Interactive Soil Bacterium *Burkholderia terrae* BS001-A Plethora of Outstanding Interactive Capabilities Unveiled. *Genome Biology and Evolution* 6, 1652–68.

Haq, I.U., Dini-Andreote, F. & van Elsas, J.D. 2017. Transcriptional Responses of the Bacterium *Burkholderia terrae* BS001 to the Fungal Host *Lyophyllum* sp. Strain Karsten under Soil-Mimicking Conditions. *Microbial Ecology* 73, 236–252.

Hiscox, J., Savoury, M., Johnston, S.R., Parfitt, D., Müller, C.T., Rogers, H.J. & Boddy, L. 2016. Location, location, location: priority effects in wood decay communities may vary between sites. *Environmental Microbiology* 18, 1954–1969.

Hiscox, J., Savoury, M., Toledo, S., Kingscott-Edmunds, J., Bettridge, A., Al Waili, N. & Boddy, L. 2017. Threesomes destabilise certain relationships: multispecies interactions between wood decay fungi in natural resources. *FEMS Microbiology Ecology* 93, fix014.

Hover, T., Maya, T., Ron, S., Sandovsky, H., Shadkchan, Y., Kijner, N., Mitiagin, Y., Fichtman, B., Harel, A., Shanks, R.M.Q., Bruna, R.E., García-Véscovi, E. & Osherov, N. 2016. Analysis of mechanisms of bacterial (*Serratia marcescens*) attachment, migration and killing of fungal hyphae. *Applied and Environmental Microbiology* 82, AEM.04070-15.

Ihrmark, K., Bödeker, I.T.M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K.E. & Lindahl, B.D. 2012. New primers to amplify the fungal ITS2 region - evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiology Ecology* 82, 666–677.

Johnston, S.R., Boddy, L. & Weightman, A.J. 2016. Bacteria in decomposing wood and their interactions with wood-decay fungi. *FEMS Microbiology Ecology* 92, fiw179.

Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lücking, R., Martín, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson,

M., Põldmaa, K., Saag, L., Saar, I., Schüßler, A., Scott, J.A., Senés, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M. & Larsson, K.-H. 2013. Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology* 22, 5271–5277.

Lane, D.J., Pace, B., Olsen, G.J., Stahl, D.A., Sogin, M.L. & Pace, N.R. 1985. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *PNAS* 82, 6955–9.

Leben, C. 1984. Spread of Plant Pathogenic Bacteria with Fungal Hyphae. *The American Phytopathological Society* 74, 176–83.

Lenth, R. V. 2016. Least-Squares Means: The R Package *lsmeans*. *Journal of Statistical Software* 69, 1–33.

Nazir, R., Boersma, F.G.H., Warmink, J.A. & van Elsas, J.D. 2010. *Lyophyllum* sp. strain Karsten alleviates pH pressure in acid soil and enhances the survival of *Variovorax paradoxus* HB44 and other bacteria in the mycosphere. *Soil Biology and Biochemistry* 42, 2146–2152.

Nazir, R., Zhang, M., de Boer, W. & van Elsas, J.D. 2012. The capacity to comigrate with *Lyophyllum* sp. strain Karsten through different soils is spread among several phylogenetic groups within the genus *Burkholderia*. *Soil Biology and Biochemistry* 50, 221–233.

Nazir, R., Mazurier, S., Yang, P., Lemanceau, P. & van Elsas, J.D. 2017. The ecological role of type three secretion systems in the interaction of bacteria with fungi in soil and related habitats is diverse and context-dependent. *Frontiers in Microbiology* 8, 38.

Nazir, R., Tazetdinova, D.I. & van Elsas, J.D. 2014. *Burkholderia terrae* BS001 migrates proficiently with diverse fungal hosts through soil and provides protection from antifungal agents. *Frontiers in Microbiology* 5, 1–10.

Oren, A. & Garrity, G.M. 2015. List of new names and new combinations previously effectively, but not validly, published. *International Journal of Systematic and Evolutionary Microbiology* 65, 2017–2025.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & Team, R.C. 2016. *nlme*: Linear and Nonlinear Mixed Effects Models.

R Development Core Team, R. 2011. R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing*.

RStudio Team, R. 2016. RStudio: Integrated Development for R.

Sarkar, D. 2008. Lattice*: Multivariate Data Visualization with R.*, Springer. New York.

Sawana, A., Adeolu, M. & Gupta, R.S. 2014. Molecular signatures and phylogenomic analysis of the genus *Burkholderia*: proposal for division of this genus into the emended genus *Burkholderia* containing pathogenic organisms and a new genus *Paraburkholderia* gen. nov. harboring env. *Frontiers in Genetics* 5, 429.

Schulz-Bohm, K., Tyc, O., de Boer, W., Peereboom, N., Debets, F., Zaagman, N., Janssens, T.K.S. & Garbeva, P. 2017. Fungus-associated bacteriome in charge of their host behavior. *Fungal Genetics and Biology* 102, 38–48.

Seigle-Murandi, F., Guiraud, P., Croize, J., Falsen, E. & Eriksson, K.L. 1996. Bacteria Are Omnipresent on *Phanerochaete chrysosporium* Burdsall. *Applied and Environmental Microbiology* 62, 2477–2481.

Simon, A., Hervé, V., Al-Dourobi, A., Verrecchia, E. & Junier, P. 2017. An *in situ* inventory of fungi and their associated migrating bacteria in forest soils using fungal highway columns. *FEMS Microbiology Ecology* 93, fiw217.

Stopnisek, N., Zühlke, D., Carlier, A., Barberán, A., Fierer, N., Becher, D., Riedel, K., Eberl, L. & Weisskopf, L. 2015. Molecular mechanisms underlying the close association between soil *Burkholderia* and fungi. *The ISME Journal* 10, 253–264.

Warmink, J.A., Nazir, R., Corten, B. & van Elsas, J.D. 2011. Hitchhikers on the fungal highway: The helper effect for bacterial migration via fungal hyphae. *Soil Biology and Biochemistry* 43, 760–765.

Warmink, J.A. & van Elsas, J.D. 2009. Migratory response of soil bacteria to *Lyophyllum* sp. strain Karsten In soil microcosms. *Applied and Environmental Microbiology* 75, 2820–2830.

Webster, G., Mullins, A.J., Bettridge, A.S., Jones, C., Cunningham-Oakes, E., Connor, T.R., Parkhill, J. & Mahenthiralingam, E. 2019. The Genome Sequences of Three Paraburkholderia sp. Strains Isolated from Wood-Decay Fungi Reveal Them as Novel Species with Antimicrobial Biosynthetic Potential.

Microbiology Resource Announcements 8, e00778-19.

Weisburg, W.G., Barns, S.M., Pellettier, D.A., Lane, D.J., Pelletier, D.A. & Lane, D.J. 1991. 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology* 173, 697–703.

Wichmann, G., Sun, J., Dementhon, K., Glass, N.L. & Lindow, S.E. 2008. A novel gene, phcA from *Pseudomonas syringae* induces programmed cell death in the filamentous fungus *Neurospora crassa*. *Molecular Microbiology* 68, 672–689.

Wickham, H. 2009. *ggplot2*: Elegant Graphics for Data Analysis.

Wickham, H. & Francois, R. 2016. *dplyr*: A Grammar of Data Manipulation.

Yang, P., Zhang, M., Warmink, J.A., Wang, M. & van Elsas, J.D. 2016. The type three secretion system facilitates migration of *Burkholderia terrae* BS001 in the mycosphere of two soil-borne fungi. *Biology and Fertility of Soils* 52, 1037–1046.

Yang, P., Oliveira da Rocha Calixto, R. & van Elsas, J.D. 2018. Migration of Paraburkholderia terrae BS001 Along Old Fungal Hyphae in Soil at Various pH Levels. *Microbial Ecology* 76, 443–452.

Yang, P., Zhang, M. & van Elsas, J.D. 2017. Role of flagella and type four pili in the co-migration of *Burkholderia terrae* BS001 with fungal hyphae through soil. *Scientific Reports* 7, 2997.

Zhang, Y., Kastman, E.K., Guasto, J.S. & Wolfe, B.E. 2018. Fungal networks shape dynamics of bacterial dispersal and community assembly in cheese rind microbiomes. *Nature Communications* 9.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A. & Smith, G.M. 2009. *Mixed effects models and extensions in ecology with R*, Springer Verlag. New York.

Figure 1 Extent of *Paraburkholderia* migration along hyphae of wood-decay fungi on 2% malt agar and 10°C and 20°C. Each point represents the maximum radial extent of bacterial migration on one replicate, at the time the fungus reached the edge of the plate. Shape and colour of points indicates bacterial strain identity.

Figure 2 Mycelial extension rates of ten wood-decay fungi on 2% malt agar in the presence and absence of *Paraburkholderia*. (a) Extension rates at 20°C; (b) extension rates at 10°C.

Figure 3 Outcomes of competitive interactions between wood decay basidiomycetes on 2% malt agar at 20°C in the presence and absence of *Paraburkholderia*. Interactions are scored on a metric that combines outcome of interaction and the time taken to reach a conclusion, where a fast win scores higher than a slow one, and a fast loss scores lower than a slow one. Error bars represent standard deviation.

Figure 4 Outcomes of competitive interactions between wood decay basidiomycetes on 2% malt agar at 10°C in the presence and absence of *Paraburkholderia*. Interactions are scored on a metric that combines outcome of interaction and the time taken to reach a conclusion, where a fast win scores higher than a slow one, and a fast loss scores lower than a slow one. Error bars represent standard deviation.

Figure 5 *Paraburkholderia* sp. BCC1885 inducing localised pigment production in *S. hirsutum*. Another replicate is provided for comparison in which the bacterium did not induce pigmentation.

Table 1 Fungal strains used to assess the effect of three migratory bacteria on fungal extension rates over agar.

Fungus	Strain	Abbreviation	Phylum	Successional stage	Source
Biscogniauxia nummularia	BxnFF1	Bxn	Ascomycota	Primary	Fruit body isolation
Vuilleminia comedens	VcWVJH1	Vc	Basidiomycota	Primary	Beech wood isolation
Bjerkandera adusta	BaSS1	Ba	Basidiomycota	Early-mid secondary	Fruit body isolation
Stereum hirsutum	ShSS1	Sh	Basidiomycota	Early-mid secondary	Fruit body isolation
Trametes versicolor	TvFPxH	Tv	Basidiomycota	Early-mid secondary	Lab cross
Hypholoma fasciculare	HfABWS1	Hf1	Basidiomycota	Late secondary/ tertiary	Cord isolation
Hypholoma fasciculare	HfGTWV2	Hf2	Basidiomycota	Late secondary/ tertiary	Fruit body isolation
Phanerochaete velutina	Pv29	Pv	Basidiomycota	Late secondary/ tertiary	Beech wood isolation
Phanerochaete sp.	PW271	Psp	Basidiomycota	Late secondary/ tertiary	Cord isolation
Resinicium bicolor	Rb1	Rb	Basidiomycota	Late secondary/ tertiary	University of Aberdeen

All strains are from the Cardiff University Culture Collection unless otherwise stated. Phanerochaete sp. PW271 and H. fasciculare HfABWS1 were both newly isolated during the course of this study.

Table 2 *Post-hoc* comparisons of mycelial extension rate for wood-decay fungi inoculated with fungal-migratory *Paraburkholderia*.

Fungus	**Bacterium**	**Coefficient**	**Std error**	**DF**	***t***	***P***
Ba	BCC1884	-0.346	0.508	355	-0.682	>0.999
	BCC1885	-0.050	0.512	355	-0.097	>0.999
	BCC1886	0.453	0.508	355	0.893	>0.999
Bxn	BCC1884	0.084	0.489	355	0.173	>0.999
	BCC1885	-0.226	0.489	355	-0.463	>0.999
	BCC1886	-0.718	0.489	355	-1.47	>0.999
Hf1	BCC1884	-1.59	0.504	355	-3.16	0.395
	BCC1885	-1.21	0.504	355	-2.39	0.930
	BCC1886	**-2.20**	**0.481**	**355**	**-4.58**	**0.004**
Hf2	BCC1884	0.177	0.502	355	0.353	>0.999
	BCC1885	0.042	0.502	355	0.083	>0.999
	BCC1886	0.050	0.516	355	0.097	>0.999
Psp	**BCC1884**	**2.57**	**0.528**	**355**	**4.87**	**0.001**
	BCC1885	**3.21**	**0.543**	**355**	**5.91**	**<0.001**
	BCC1886	**2.67**	**0.548**	**355**	**4.87**	**0.001**
Pv	**BCC1884**	**2.24**	**0.527**	**355**	**4.25**	**0.016**
	BCC1885	**4.10**	**0.533**	**355**	**7.70**	**<0.001**
	BCC1886	**3.75**	**0.570**	**355**	**6.58**	**<0.001**
Rb	BCC1884	0.515	0.610	355	0.845	>0.999
	BCC1885	0.955	0.610	355	1.57	>0.999
	BCC1886	1.35	0.610	355	2.21	0.974
Sh	BCC1884	0.177	0.538	355	0.329	>0.999
	BCC1885	-0.036	0.538	355	-0.067	>0.999
	BCC1886	-0.088	0.538	355	-0.164	>0.999
Tv	BCC1884	-0.155	0.525	355	-0.295	>0.999

	BCC1885	-0.144	0.525	355	-0.274	>0.999
	BCC1886	-1.062	0.525	355	-2.02	0.994
Vc	BCC1884	-0.136	0.533	355	-0.254	>0.999
	BCC1885	-0.324	0.526	355	-0.616	>0.999
	BCC1886	-0.921	0.525	355	-1.75	>0.999

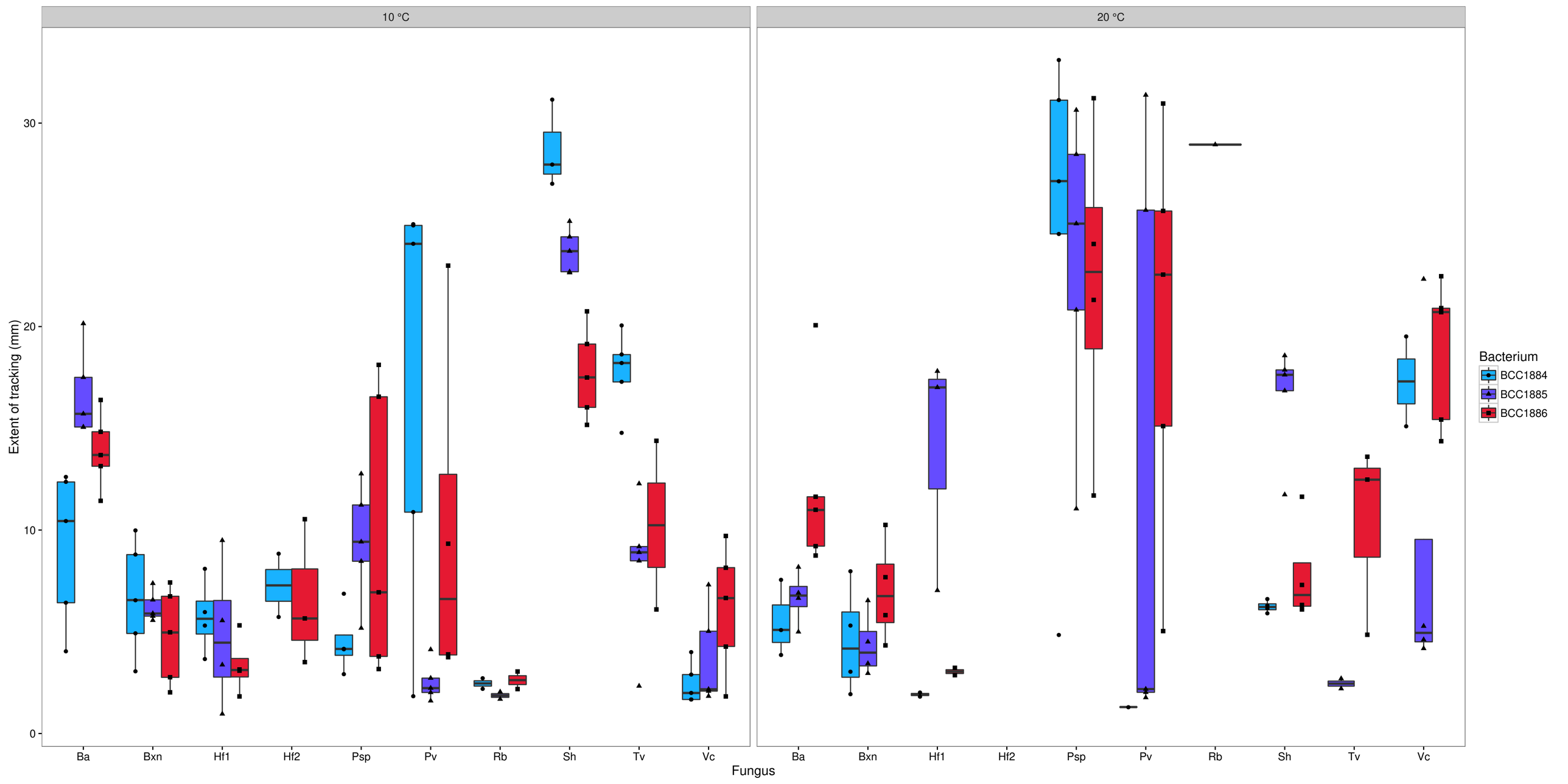
Comparisons are derived from a linear mixed effects model. Each row shows the effects of a given bacterium-fungus combination compared to the bacteria-free control for that fungus. The coefficient gives the magnitude and direction of change (negative values indicate an increase in extension rate relative to the control, positive values indicate a decrease). P value adjustment: Tukey method for comparing a family of 40 estimates (not all comparisons are shown). Significant terms are shown in bold. All numbers are given to three significant figures. Fungal abbreviations are given in Table 1.

Table 3 Model output for outcomes of competitive interactions between wood-decay fungi and the effect of inoculating each competitor with fungal-migratory *Paraburkholderia*.

Term	**Coefficient**	**Std error**	***t***	***Pr(>|t|)***
(Intercept)	**-0.275**	**0.027**	**-10.3**	**<0.001**
Ba	**0.448**	**0.031**	**14.6**	**<0.001**
Sh	-0.021	0.024	-0.905	0.366
Psp	**-0.278**	**0.031**	**-9.07**	**<0.001**
Pv	-0.040	0.026	-1.57	0.115
Ba-BCC1884	-0.041	0.048	-0.842	0.400
Sh-BCC1884	0.018	0.048	0.382	0.703
Psp-BCC1884	**-0.205**	**0.048**	**-4.25**	**<0.001**
Pv-BCC1884	0.033	0.048	0.691	0.490
Tv-BCC1884	0.014	0.048	0.292	0.771
Ba-BCC1886	-0.025	0.048	-0.525	0.600
Ba-BCC1885	**-0.122**	**0.048**	**-2.54**	**0.011**
Sh-BCC1885	-0.053	0.048	-1.09	0.275
Psp-BCC1885	**-0.296**	**0.049**	**-6.04**	**<0.001**
Pv-BCC1885	**-0.111**	**0.048**	**-2.31**	**0.021**
Tv-BCC1885	0.073	0.049	1.49	0.137
Sh-BCC1886	0.001	0.048	0.012	0.990
Psp-BCC1886	**-0.602**	**0.048**	**-12.5**	**<0.001**
Pv-BCC1886	-0.068	0.048	-1.41	0.159
Tv-BCC1886	0.046	0.049	0.942	0.346
Temp20	**0.157**	**0.022**	**7.22**	**<0.001**

The fungal identity matrix is calculated with zero-sum contrasts; the estimate for Tv may be obtained by subtracting the coefficients for the other fungi. Positive or negative scores show a winning or losing tendency, respectively. Each subsequent row shows the effects of a given bacterium-fungus combination, averaged across all opponents. The coefficient gives the magnitude and direction of

change (negative values show a decrease in competitive ability, positive values an increase). Null deviance = 213.96 on 986 degrees of freedom; residual deviance = 112.33 on 966 degrees of freedom; R^2 *= 0.47. Significant terms are shown in bold. All numbers are given to three significant figures. Fungal abbreviations are given in Table 1.*


10 °C
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Ba
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Psp
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Tv
Vc
Fungus
Bacterium
BCC1884
BCC1885
BCC1886

Ba
Bxn
Hf1
Hf2
Psp
Pv
Rb
Sh
Tv
Vc
Growth rate (mm/day)
6
4
2
BCC1884
BCC1885
BCC1886
Control
(a) Treatment (20°C)
Ba
Bxn
Hf1
Hf2
Psp
Pv
Rb
Sh
Tv
Vc
Growth rate (mm/day)
2
1
BCC1884
BCC1885
BCC1886
Control
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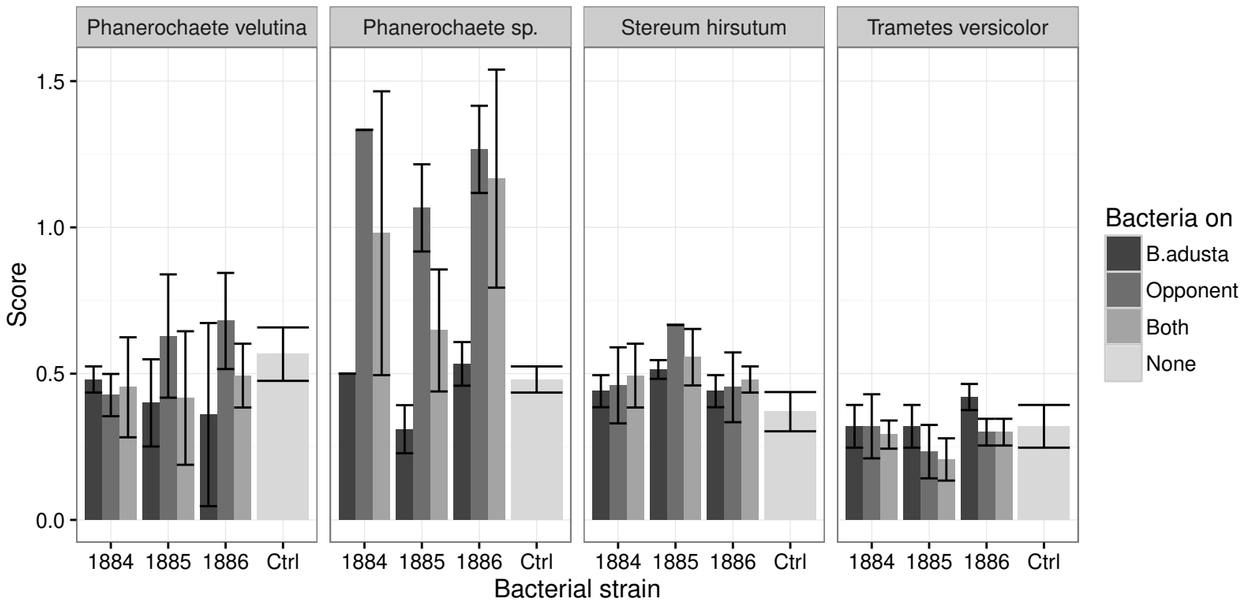
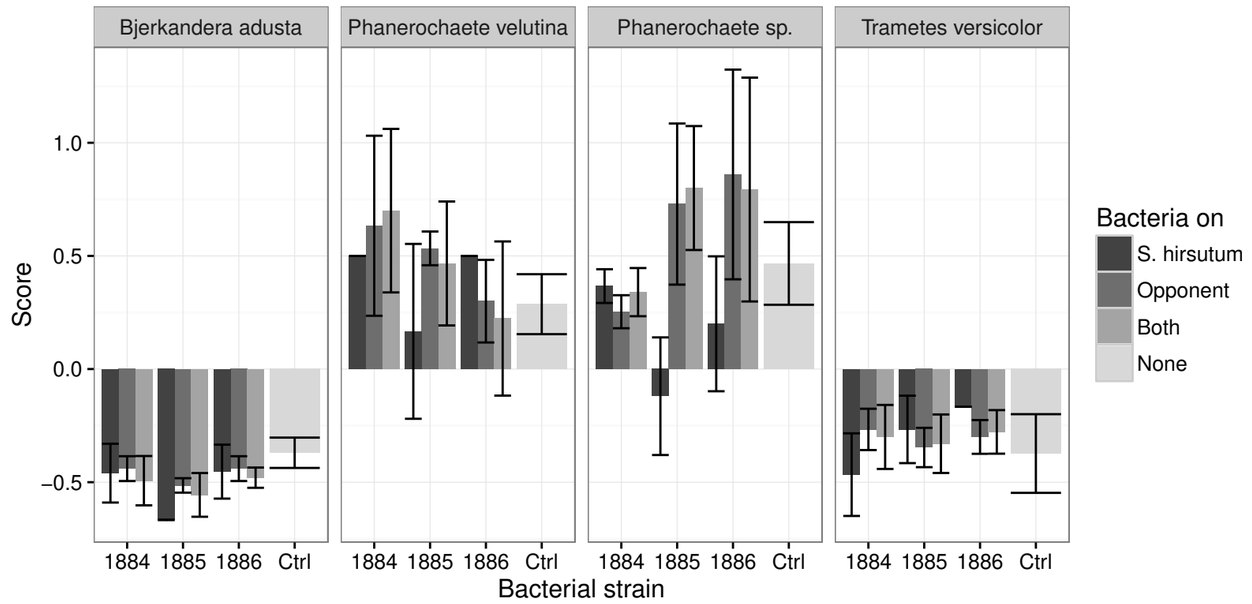
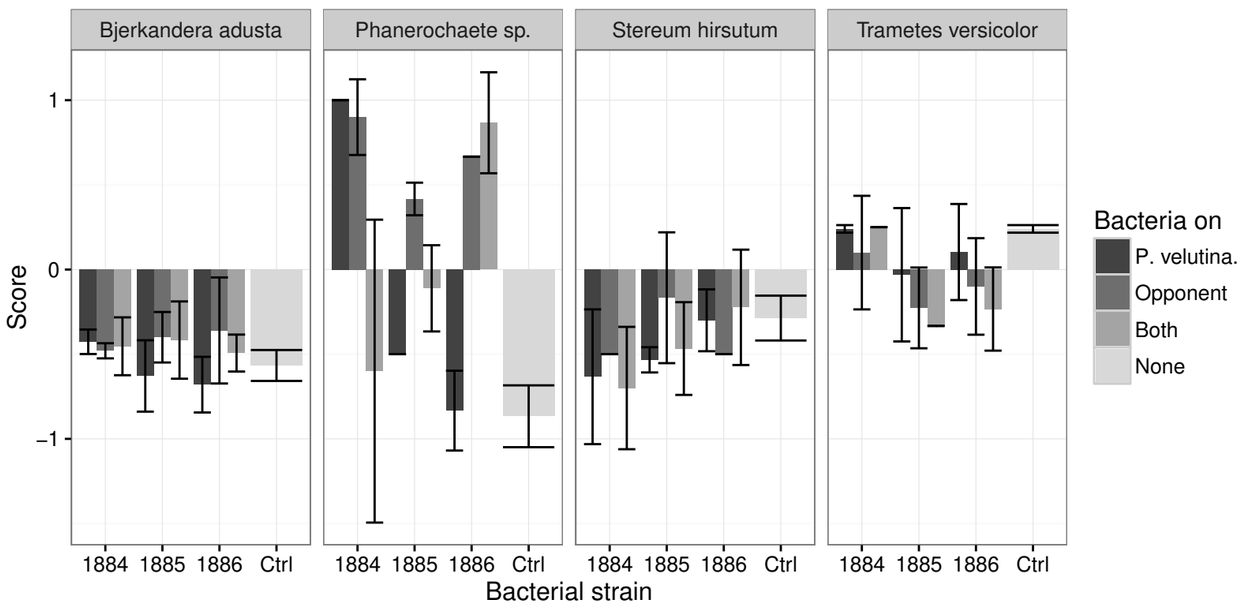
Bjerkandera adusta



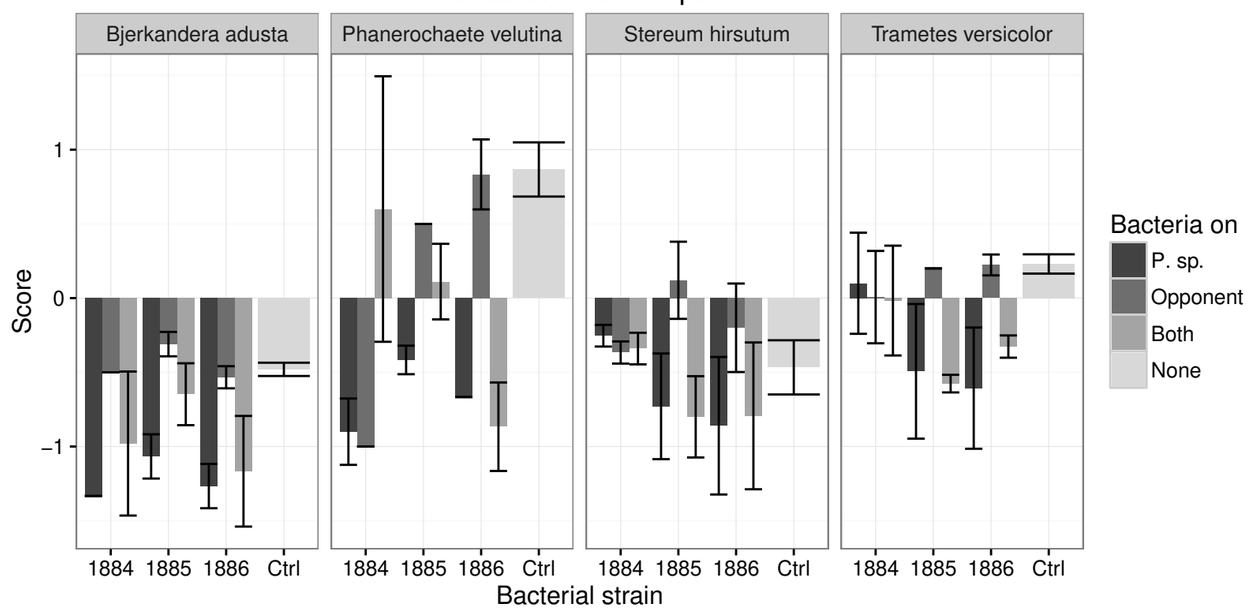
Stereum hirsutum



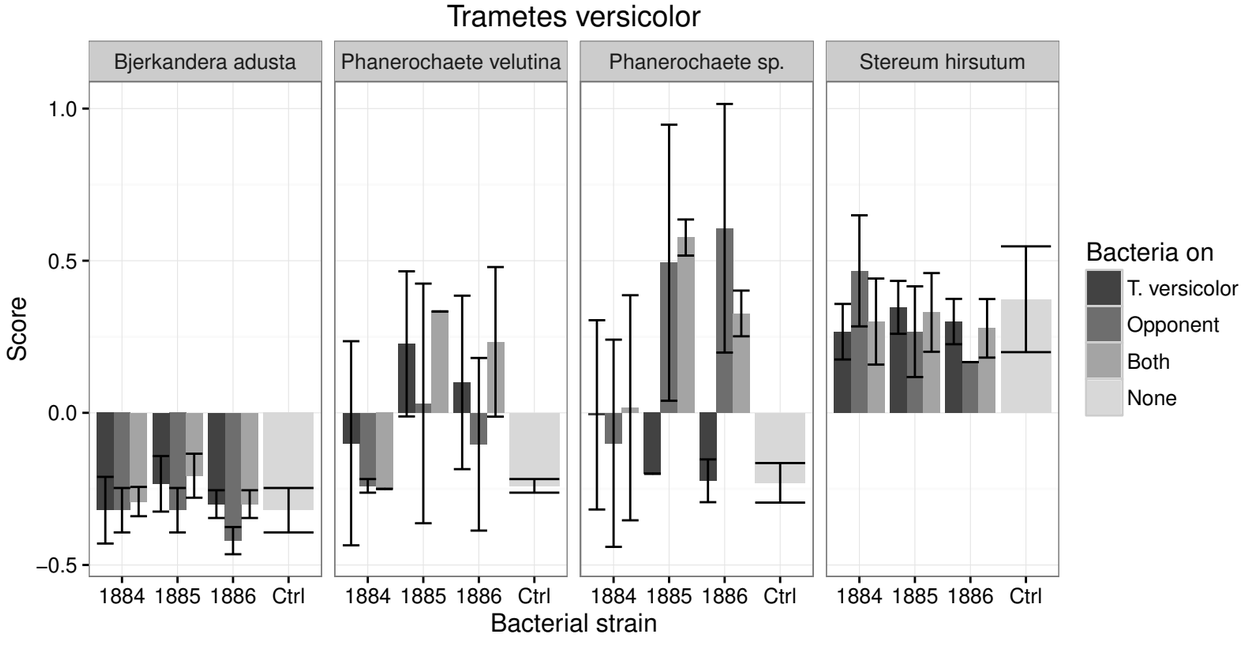
Phanerochaete velutina



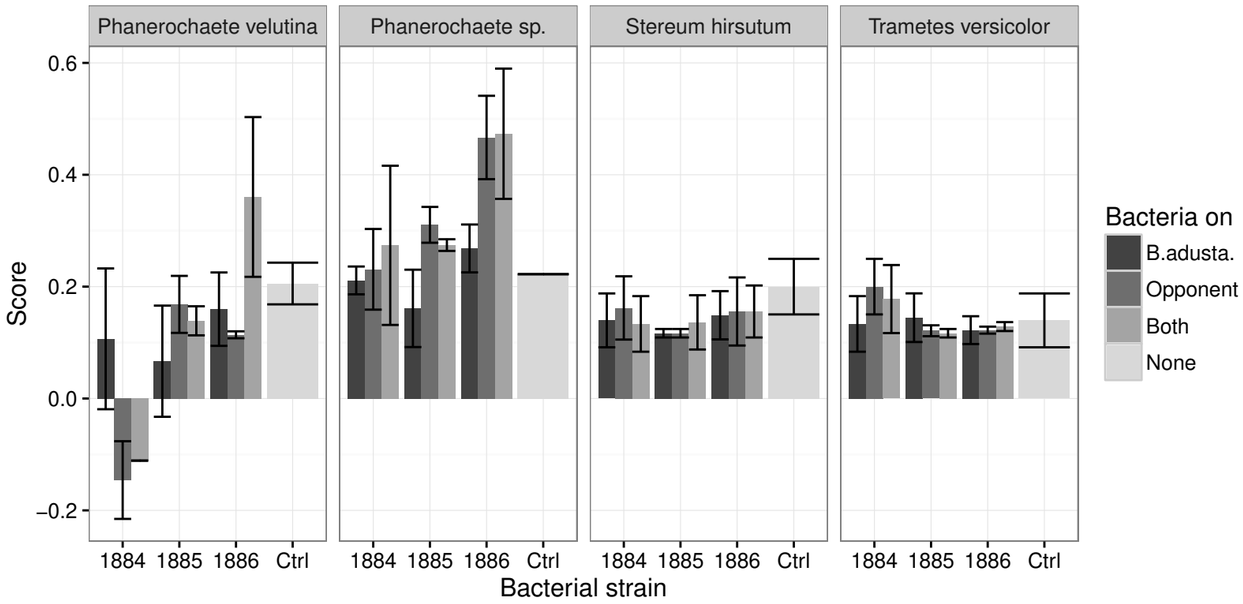
Phanerochaete sp.



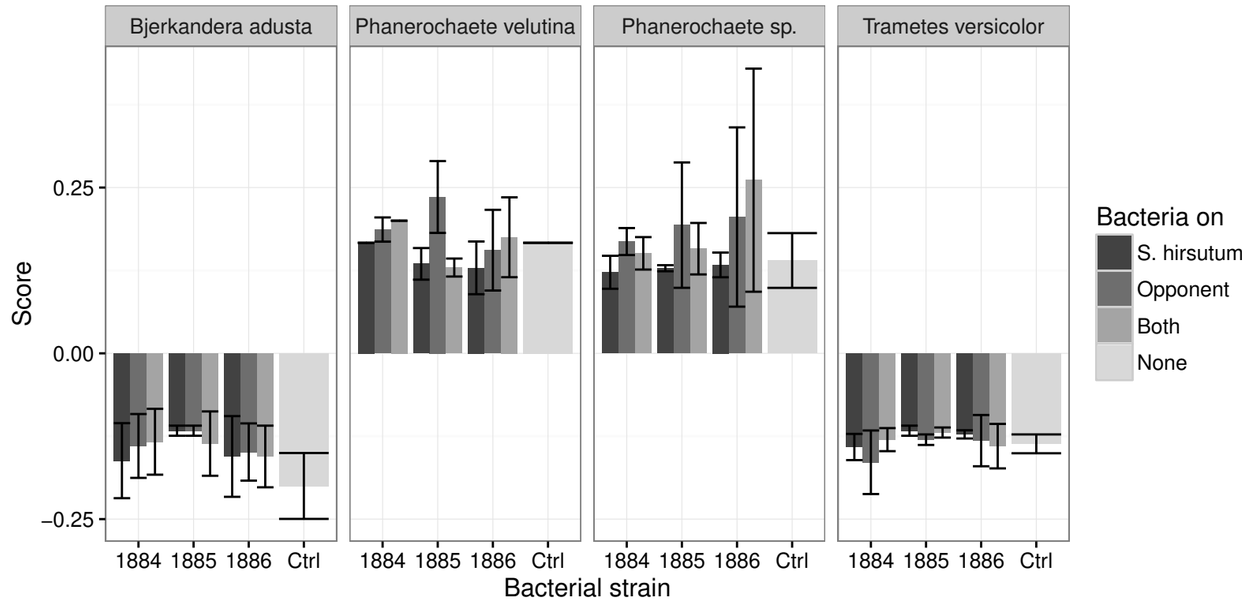
Trametes versicolor



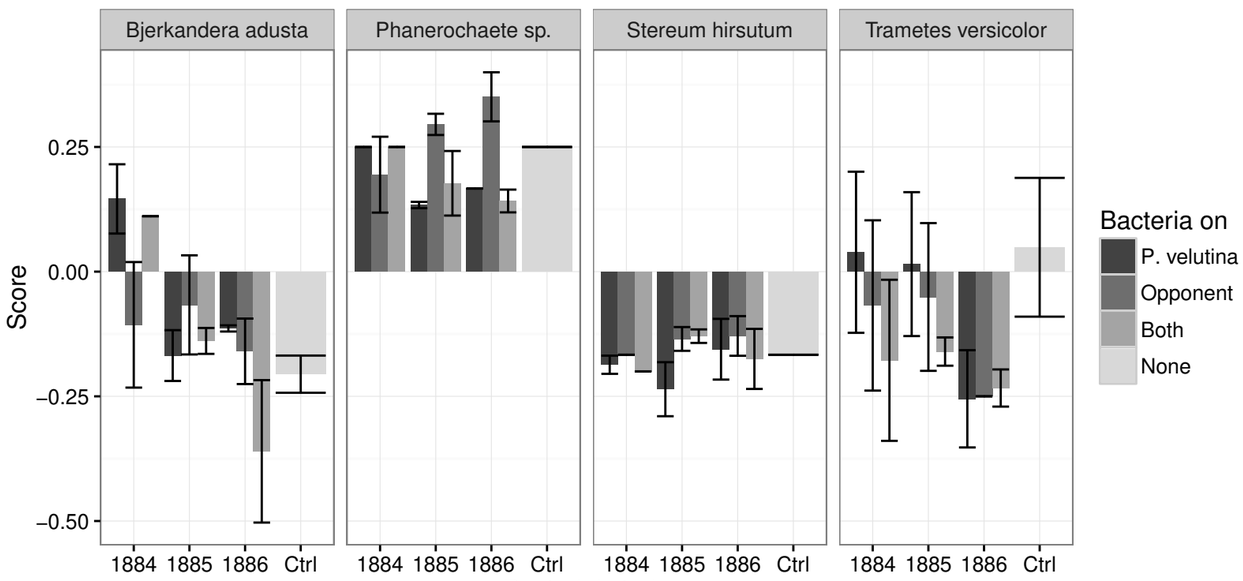
Bjerkandera adusta



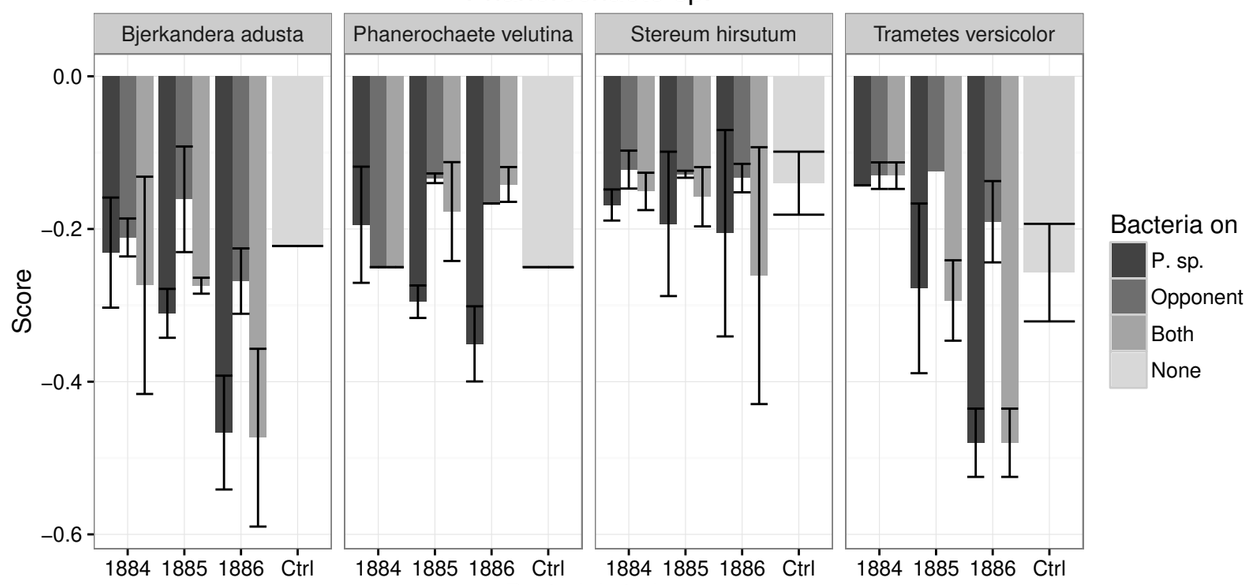
Stereum hirsutum



Phanerochaete velutina



Phanerochaete sp.



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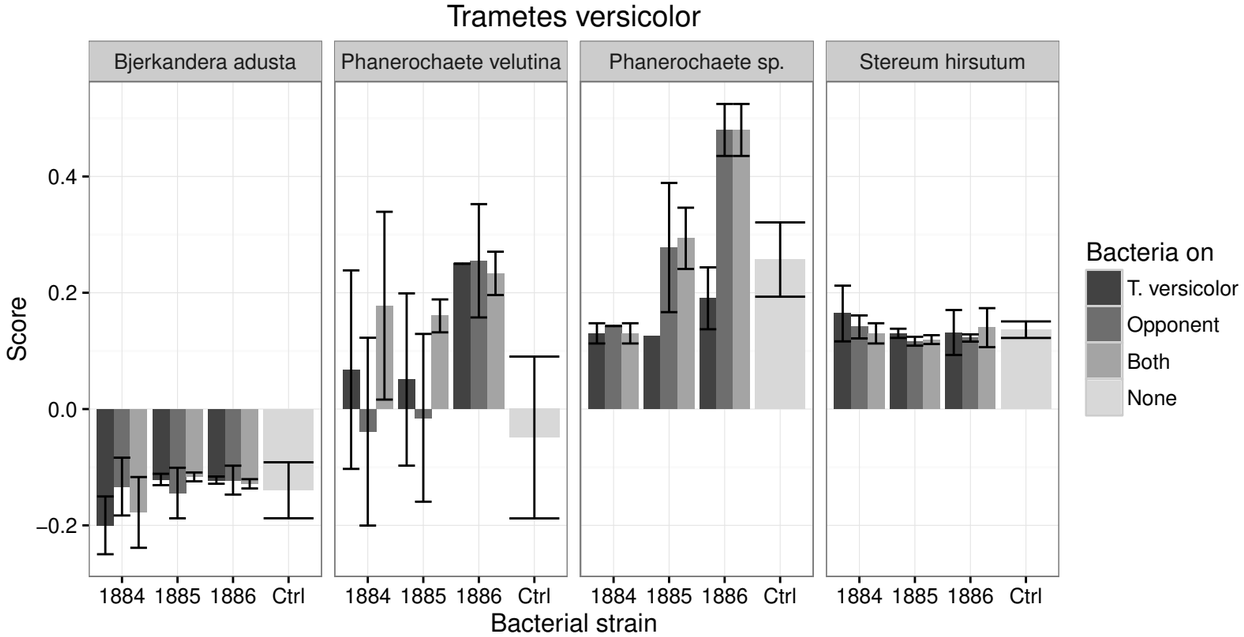

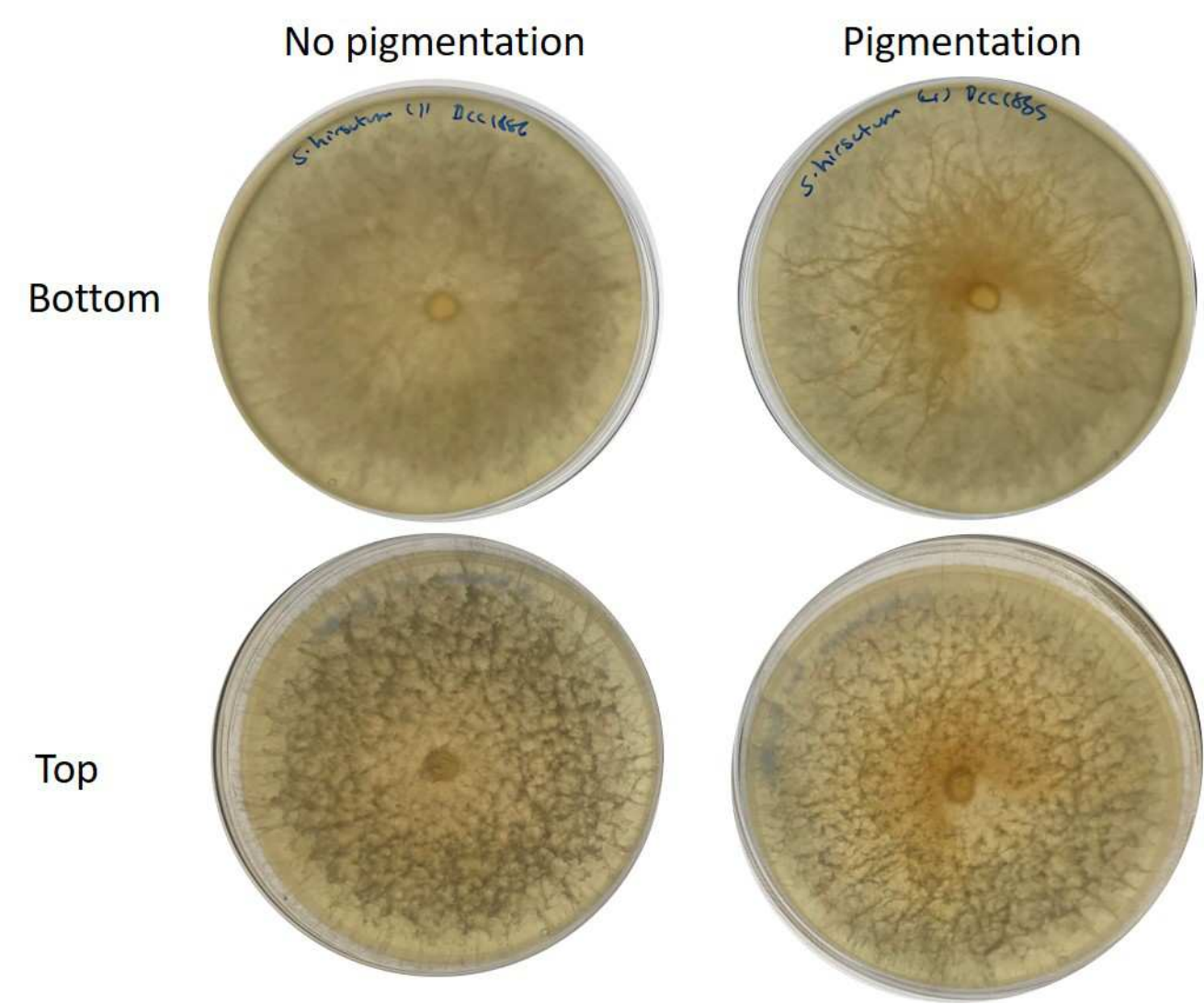
No pigmentation
Pigmentation
Bottom
Top

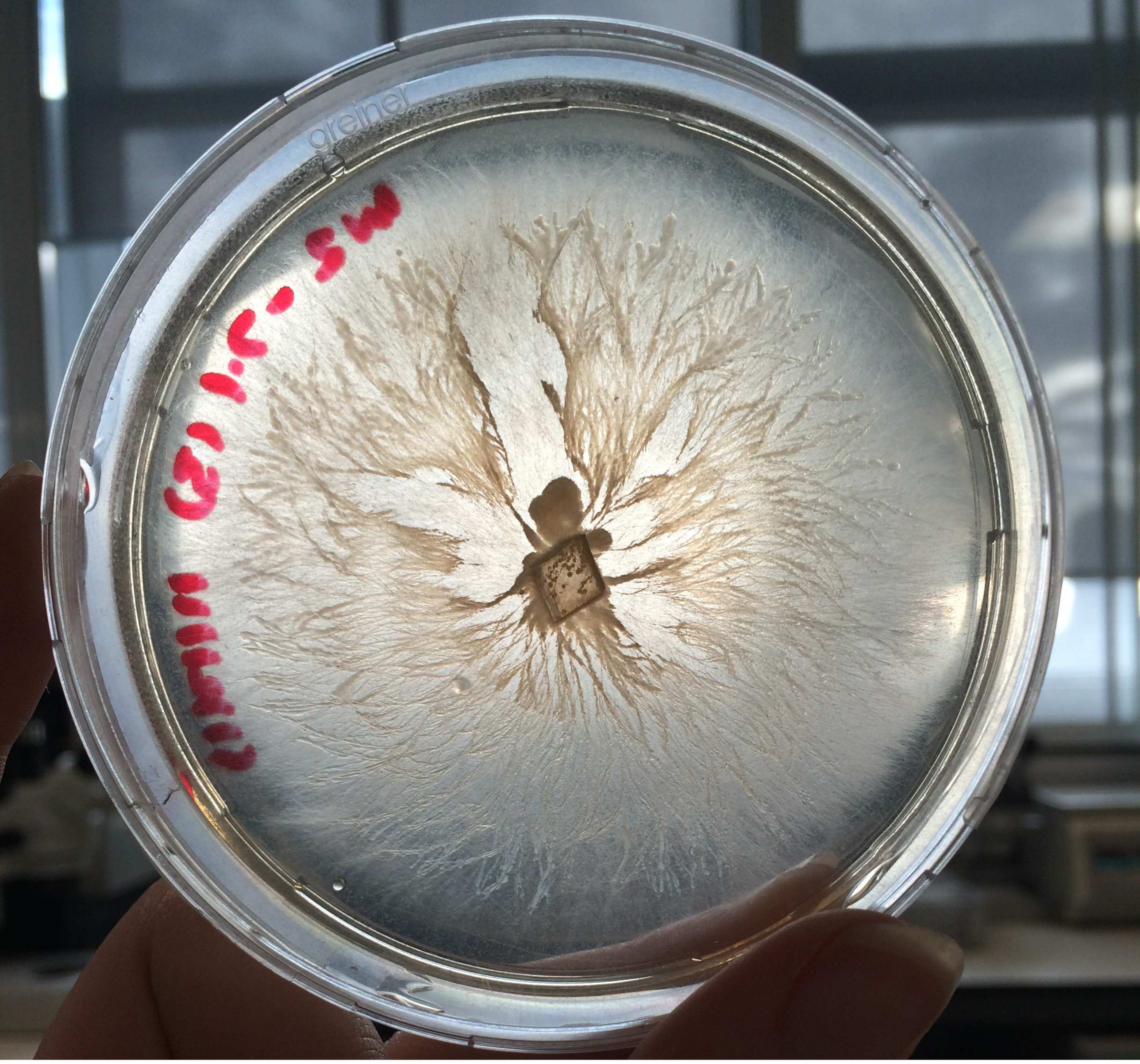

Figure S1 *Paraburkholderia* sp. BCC1884 migrating over hyphae of *Phanerochaete* sp. PW271. Note the distinctive linear pattern of colonies following hyphae, as distinct from the rounded colonies at the central inoculation site.

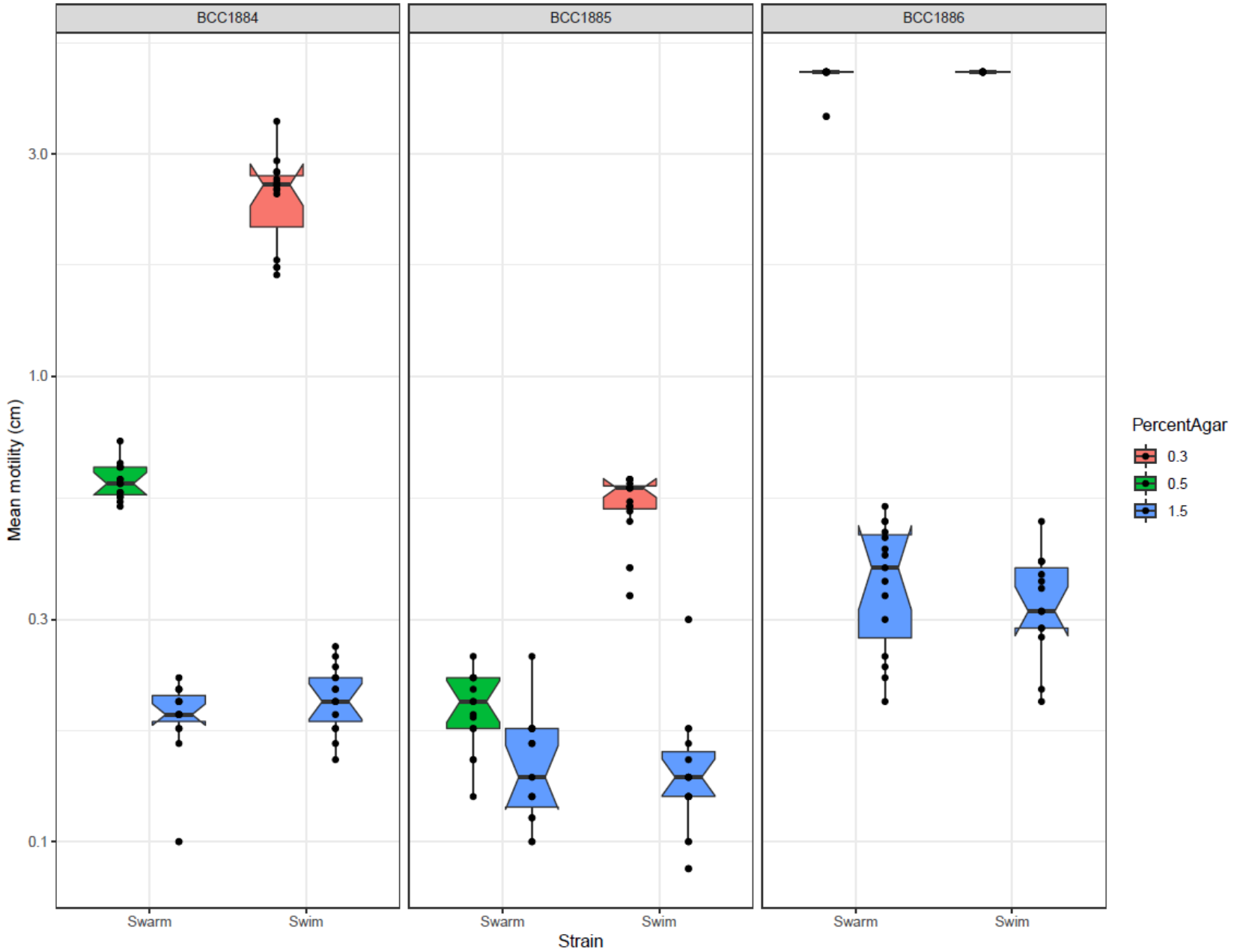

Fig S2. Swarming and swimming motility of the three *Paraburkholderia* isolates in the absence of mycelium and at different agar concentrations. Note the *y* axis is on a log scale.